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ANNUAL REPORT

R&T CODE: 441m804---01

DATE: 15 DEC 1988

FIRST ANNUAL REPORT ON CONTRACT N00014-88-K-0078

PRINCIPAL INVESTIGATOR: David P. Corey

CONTRACTOR: Massachusetts General Hospital

CONTRACT TITLE: Adaptation and Regulation of the Transduction Mechanism in Vestibular Hair Cells

START DATE: 15 December 1987

The ~~RESEARCH OBJECTIVES~~ ^{AND} To characterize the time and displacement dependence of adaptation in hair cells; to localize the site of action of calcium and determine its calcium specificity; to identify and localize calcium-binding and structural proteins in stereocilia that may mediate calcium's effect.

PROGRESS (Year 1): Work on this project has gone extremely well: the time and displacement dependence objective is done, and a manuscript has been submitted to the *Journal of Neuroscience*. The results indicate that the adaptation process involves the movement of the attachment point of the spring attached to transduction channels; this may be a movement of one end of the morphologically-defined "tip links". They also suggest that there are two separate processes in the adaptation: One is a slipping or relaxing process that is linearly proportional in rate to the displacement, indicating that the rate may depend on tension in the tip link. The other is a climbing or tensioning process that is largely independent of displacement, suggesting an intrinsic climbing rate. Incidentally, the rate and final tension of the element are nearly identical to that of myosin moving on actin.

We also have a good measure of the calcium dependence, using the microphonic preparation, and have made simple measurements of the cation specificity. Generally, the relaxation rate is increased by high calcium, but the tensioning rate is less sensitive. The binding site appears to be specific for calcium, as magnesium, strontium or barium inhibit calcium's action.

Or, another project, patch clamp experiments have suggested that the site of calcium action is inside the tips of the stereocilia (manuscript in press in *Proc. Natl. Acad. Sci*). While this should be confirmed with more direct methods, at this point all results are consistent with some molecular motor intimately associated with the transduction apparatus at the tips of the stereocilia.

Immunohistochemical experiments have indicated that calmodulin is inside the tips of the stereocilia, as well as in the cell body. There is also calbindin throughout the cell, based on immunohistochemistry. More calcium-binding proteins are being discovered every day, however, and it will be an interesting task to determine which of the many candidates is actually mediating the calcium dependence.

WORK PLAN (Year 2): We feel that a complete understanding of the adaptation process will only come about through an eventual identification and understanding of the specific proteins involved. Consequently, we will put a lot of effort into developing a method to purify stereocilia, and to separate the proteins in them with one- and two-dimensional gel electrophoresis. It may be possible to identify and localize these proteins with a combination of immunoblots and immunocytochemistry. The ultimate goal (which may not be within the scope of this grant), would be to determine the identity of a particular protein, localize it within the cilium, and understand its physiological role in the transduction or adaptation.

For year 2, then, efforts will focus on the biochemical separation and identification of stereociliary proteins, and on immunohistochemical localization with antibodies to known proteins. Physiology experiments will continue in order to finish up some of the first year objectives.

INVENTIONS (Year 1): While we have modified some standard techniques in novel ways, none would be considered patentable inventions.

PUBLICATIONS AND REPORTS (first year):

Assad, J.A., Hacohen, N., and Corey, D.P. (1989) Voltage dependence of adaptation and active bundle movement in bullfrog saccular hair cells. *Proc. Natl. Acad. Sci. USA* (in press).

Hacohen, N., Assad, J.A., Smith, W.J., and Corey, D.P. (1989) Regulation of tension on hair-cell transduction channels: Displacement and calcium dependence. *J. Neurosci.* (submitted)

TRAINING ACTIVITIES: John Assad, a graduate student in the Neurobiology Program at Harvard Medical School, has been working on the physiology of this project since its beginning. Gordon Shepherd and Philip Huang, both MD/PhD students at Harvard Medical School, have started to work on biochemical aspects of the project. All are exceptionally good and are key to the success of the project.

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